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Patient safety and quality improvement programmes

Patient safety and quality improvement programmes are under discussion in many countries, including Finland. Here the Ministry of Social Affairs and Health has outlined its Finnish Patient Safety Strategy for 2009–2013. One of the key elements and aims in this discussion is to achieve an open, non-punitive patient safety culture in which organizations as well as individuals learn from mistakes – and do not repeat the same ones so often. This should lead to reduced rates of medical errors and better patient satisfaction, bearing in mind that improving quality saves not only lives, but also money.

One of the tools for achieving this goal is to introduce a Registry of Medical Errors – a broad, voluntary, easy-to-use system to collect and analyse incidents and errors that could harm patients. This idea takes advantage of what has been learned about safety by the airline industry, where similar reporting systems have been in place for many years. The idea of collecting data and learning from each other is also very like what we are used to in our External Quality Assessment (EQA) Schemes. In that sense, I feel that this kind of new tool to improve patient safety and work to reduce preventable injuries in health care is similar to what we offer clinical laboratories. The new safety and quality outcome reporting solutions under development and educational EQA programmes should therefore be seen as complementing each other. EQA providers could benefit from the new reporting and data collection solutions, but I believe health care and patient safety organizations could also learn quite a lot from EQA providers and quality work done in clinical laboratories over many years. We have learned that data alone are not enough. There is a continuous need for education about how to get value and benefits from the collected data and summary reports, and how to improve procedures, quality of equipment and methods, quality of diagnosis and care, etc.

To achieve patient safety and improved quality in patient care, we need qualified health care professionals and also active, well-informed patients together utilizing the possibilities available for diagnostics, care, disease follow-up and prevention. Quality measurement should not be limited to measuring technical or analytical quality. Issues such as patient-centred operations, timeliness, safety, effectiveness, efficiency and fairness should be the focus of attention. Although Labquality’s EQA service, which is described in this issue of Labquality News, has been developed mostly for use in laboratory medicine, we see our work as part of broader ambitious health care objectives. Our mission is to promote patient safety by improving quality.
EQA Schemes in Laboratory Medicine
Cooperation on quality for 39 years

Pilot EQA Schemes were started by laboratory professionals in the 1960s and became more regular when an EQA organization was founded in 1971. From the very beginning Labquality has been fortunate to have enthusiastic clinical chemists and doctors as managers, board members, survey experts and working group members. Utilizing the expertise of clinical laboratories, Labquality very quickly got a steady foothold for its EQAS in routine medicine laboratories, first throughout Finland and later in other countries.

Labquality also benefits from employing at its office a large range of creative professionals with skills in laboratory medicine, finance, logistics, communication systems, client services and public relations, among other things. The surveys could not function efficiently without their important contribution.

Pioneer schemes

Labquality launched its EQA schemes in 1971 with General Clinical Chemistry. In subsequent years Haemoglobin (1972) and Leucocyte differential count (1973) followed. The Microbiology range was very modest in 1977, but it was ambitiously extended annually, as were schemes in Haematology and Clinical Chemistry. Laboratory instrumentation and Immunology were new in 1981, Genetics in 1993, Immunohistochemistry, Histopathology and Cytology in 1993–1995 and Andrology in 1996. In 39 years the range of EQAS has grown to nearly

An authentic and stable specimen is the most important part of the scheme.
160 schemes with 600 annual surveys.

**Heart of the Scheme**

The specimen is the most important part of the scheme. In the early days, when only a few commercial specimens were available, starting a new scheme was dependent on self-made specimens, prepared by the survey experts in laboratories. Collecting native specimens was possible for national use when the number of participants was smaller and the distances were moderate.

An authentic and stable EQA specimen available in unlimited volume is desirable but very often absolutely impossible. Only visual interpretation of a picture or a fixed and stained section on a slide enables all participants to have a stable and similar specimen. However, such a specimen omits staining technology and evaluates only part of the procedure, i.e. recognizing a finding in the picture.

EQA schemes usually monitor only the analytical phase of the procedure. Preanalytical and postanalytical phases remain outside their scope.

From the very beginning, ideally the EQA specimens and patient samples would behave in the same way in analyses. In practice, quality control material manufactured commercially may contain both human and animal-based sera, as well as other components. The aim is to keep the material as natural as possible, but generally it has been manipulated and spiked in different ways. It is also known that handling causes differences between methods, so results are compared only within each individual method group.

**Two-way communication**

In EQA schemes, cooperation between a participant and Labquality includes exchange of data and information. Labquality sends specimens and instructions, a participant returns the results and method data, and finally receives a report on performance.

Since 2006 individual result reports have been available on Labquality’s website. The service is opened with the laboratory’s client code and password.

Labquality’s aim is to give a participant the means to follow up the analytical performance. The reports show individual results compared with those in the same method group. In many reports there is also a summary of the laboratory’s results from the ten previous surveys to check long-term performance. Interpretation of results and corrective actions are the laboratory’s responsibility. The website includes quality specifications at http://www.labquality.fi/in_english/instructions/ and examples linked to introduction of a scheme to help the interpretation.

**Education in quality**

User meetings commenced in 1990 under the heading “Nordic approaches in Quality Control”, which was soon changed to “International approaches”.

In February 2011, Labquality’s 40th anniversary will begin with Labquality Days at the Helsinki Fair Centre. The programme will be published on Labquality’s website in November 2010.

The international Labquality Days are part of a large annual Quality Meeting for medical laboratories. An associated exhibition will also be arranged.

**Facts**

For nearly 40 years Labquality has been acting as an external quality assessment organizer for clinical laboratories, and for over 20 years it has operated internationally. There are now twelve EQA coordinators at the office and a hundred experts in laboratories running the six hundred annual surveys. The number of participants is 4400.

The author has been an EQA coordinator and Communications Manager at Labquality.
Labquality operates worldwide

JUHA WAHLSTEDT

Labquality’s mission is to promote patient safety by improving quality and restricting errors in laboratory diagnostics. Labquality nowadays has clients in forty countries, mainly Finland, Scandinavia, the Baltic States, Eastern Europe, Southern Europe and the Middle East. There are also a few clients in other countries all over the world, such as Australia, Canada, Costa Rica, Singapore, Thailand and the USA.

The numbers of participating countries and participating laboratories have been growing steadily year by year. The total number of participants is now over 4400. Almost the half of the participants are in the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden), but the rest of the world is growing fast. Labquality has a distributor or cooperator in 25 countries. The distributors are responsible for marketing and selling of the EQA services in their respective territories, and organizing the sample logistics together with Labquality. Client service, training and consulting about the services are divided between the distributor and Labquality case by case.

Distributors are vital for delivering dangerous goods. Some survey specimens are classified (IATA, ADR) as dangerous goods and classified (UN3373) as Biological substances, category B. Delivery of these
specimens is restricted by many airlines, national postal services and countries. Labquality therefore provides schemes that include UN3373 specimens available only via distributors.

Labquality nowadays has clients in forty countries, mainly Finland, Scandinavia, the Baltic States, Eastern Europe, Southern Europe and the Middle East. There are also a few clients in other countries all over the world, such as Australia, Canada, Costa Rica, Singapore, Thailand and the USA. The numbers of participating countries and laboratories have been growing steadily year by year. The total number of participants is now over 4400. Almost the half of the participants are in the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden), but the rest of the world is growing fast.

If there is no distributor available, laboratories can operate directly through Labquality. All the main programmes are available to direct clients, but there are several limitations on the services. It is not always possible to deliver specimens because of the very high cost (courier services) or too long delay (air mail). Microbiological schemes including UN3373 specimens are not available to direct clients as described above.

Open EQA programme
Labquality’s international programme is extensive and covers almost all expertise in laboratory medicine. Expert groups evaluate the programmes regularly, and new programmes and pilot programmes become available every year. Labquality also provides special programmes in cooperation with other EQA providers.

Labquality’s programme is very flexible and customizable for local needs. Because there is no common consensus on the frequency of EQA surveys, Labquality has decided to provide open programmes. A laboratory or a distributor can create an annual EQA programme specially for its own quality needs. That is also the most cost-effective way to use external quality assessment.

Every year Labquality sends the programme or update of the programme to all direct clients and distributors in the autumn. All clients are asked to renew their registration for the next year before the end of November. The programmes can be started at any point of the year, but participation in all the surveys is guaranteed only for clients registering before the end of November.

Distributors have their own country-customized programmes and offers. The registration cycle is basically the same as for direct clients, but there are differences in some countries. The details of programmes, registration and pricing should always be checked with the Labquality distributor.

Specimens and deliveries
Labquality’s aim is to use the best specimens available. The ideal EQA specimen resembles a real patient sample as closely as possible. Samples vary from survey to survey, covering different levels and different sample manufacturers. This ensures the quality of the scheme is high and the scheme is independent of the system or sample manufacturer.

In many cases Labquality can use real patient samples as EQA specimens, but in some cases the specimen is completely artificial for stability reasons. Because of the nature of the specimens, Labquality sends the specimens out weekly according the annual programme. In most of the programmes, specimens cannot be sent out in the beginning of the year and stored in the client laboratories before analysis. The real patient samples come from

"A laboratory or a distributor can create a tailored annual EQA programme."
a laboratory and are forwarded immediately to the participants.

EQA specimens are sent weekly to the distributors and direct clients using the fastest possible way. The distributors forward the specimens to the participants in their territory. If the analytes are labile, the specimen instructions will say that analysis is required immediately after arrival. In some cases the specimen can be stored for a short period before the analysis. In some cases where the analytes are very labile at room temperature but can be frozen, the specimens are sent out in dry ice or cooled by ice containers.

The national holidays and special seasons in forty client countries are a real challenge for the weekly specimen deliveries. Planning and cooperation with the distributors keep the client service busy around the year.

**Analysis**

The EQA specimens are meant to be used only once. The basic idea of EQA is to handle and analyse the unknown EQA sample in the same way as patient samples. To get the greatest benefit from the EQA, do not always give the EQA samples to the best trained person, always analyse the sample at the same time and in the same way as patient samples. Repeat the analysis or ask another opinion only if you would do that for a patient sample, and report the results just as you would do routinely. Only in this way you can use the EQA to evaluate your per-
formance. Analysing the specimens on time and reporting the results to Labquality on time according to the instructions is also a way of showing the quality of the performance of a laboratory.

The EQA samples are not meant to be stored and used as reference materials after the survey. In many cases the analytes are so labile that the specimen cannot be stored at all. Some samples can be stored after analysis and used afterwards as a rough control of the analysed results of the EQA survey.

Evaluation of the results

There are very different practices, recommendations and even laws to define the targets for the analytes. To cope with all these different needs, Labquality has decided to use its own target limits that are defined by Labquality’s expert groups. The target limits for the quantitative analytes are based on the biological variation of the analytes in and between individuals and the state of the art of the methods. The limits are quite tight and they are more like quality goals than limits for good and bad performance. We call our limits “educational” limits.

The most important thing is to define your own quality goals before starting to use EQA. An EQA scheme is just a meter that can help you to measure and show your performance over a long period of time. The whole EQA can be just waste of money if you have not decided the goals for your performance. You will only get only more questions than answers when you get a result which is outside Labquality’s target limits. If a laboratory has defined its own goals and has a practice for bad results, the EQA really has an effect on the system. The quality will be improved and the benefits of the EQA will be achieved.

It is also important to remember that EQA does not replace internal quality control. The IQC is as vital as the EQA for the quality system for laboratory tests.

Labquality has over a hundred experts who evaluate the survey specimens, methods and statistics. The EQA coordinator and scheme expert comment on the results and are available for support and an important part of the client service.

International cooperation and quality management

Labquality is a member of international organizations of EQA providers. The EQA organizers of Nordic countries have EQANord and in Europe there are two organizations EQUALM and EurachemPT. Several of Labquality’s distributors are EQA providers and members of previous organizations. The work at international level includes research and development projects, and harmonization of EQA terms, practices and quality issues.


Wide ownership

Labquality is an independent and impartial organization owned by the Finnish Society of Clinical Chemistry, Association of Finnish Local and Regional Authorities as well as 19 Local Hospital Districts, Finnish Medical Association, Association of Medical Service Providers and Finnish Union of Experts in Science. Labquality’s owners represent health care widely and all profits from Labquality go back into health care.

The author is a Client Relations Manager at Labquality
How to choose and use External Quality Assessment Schemes

MINNA LOIKKANEN

External quality assessment schemes (EQAS) are also known as Proficiency testing (PT) and Interlaboratory comparison testing. Laboratories can demonstrate and verify the accuracy of their measurement and testing results by participating in external quality assessment surveys (EQA) i.e. reference measurements and proficiency testing (PT). This means that an external party arranges the survey, on the basis of which it produces an objective report of the laboratory’s performance. A laboratory has to demonstrate its competence in order to meet the requirements for accreditation, to comply with authority regulations, to satisfy the needs of clients and to demonstrate the comparability of results.

Aims, challenges and benefits of proficiency testing

The general aims of proficiency testing in the field of laboratory medicine are:

- continuous improvement of quality
- maintaining a consistent standard of results between laboratories
- comparability of methods
- detection and correction of national and international problem areas.

Important challenges include the continuous improvement of the methodological and analytical knowledge of laboratory professionals and issues relating to training. Over the last few years, collaboration

Minna Loikkanen is a board member of EQALM, an association of EQA providers. PHOTO: A. SIUKOLA
with industry on methodological development has been stepped up.

A laboratory participating in EQA programme should naturally receive added value and benefits in return. Methodological performance monitoring by an external party functions as a risk management tool by providing information on the accuracy of the methods and on variation between laboratories. EQA has usually been seen as supporting internal quality control, rather than as replacing it.

The use of certified, national reference materials offers a tool for demonstrating traceability and determining deviations in results. Additionally, presenting a laboratory’s measurement uncertainty data alongside the measurement uncertainty reference value provides clear additional information on the accuracy and quality of the laboratory’s results (Fig. 1).

**Standards and criteria guiding the EQA provider**

An international standard for accreditation has been needed for a long time for EQA. Some countries have accredited or acknowledged providers in accordance with ISO/IEC Guide 43-1:1996 and ILAC-G13:2008. An accreditation standard for EQA providers, ISO 17043, was approved on 1 February 2010. This standard combines the criteria of the above-mentioned ISO/IEC Guide 43-1:1996 and ILAC-G13:2008 and clarifies certain details. The standard is intended for all organisations providing reference measurements. Internationally, the standard has faced at times severe criticism regarding its unsuitability for EQA in laboratory medicine.

When the importance of operating and quality systems was first stressed in mid-1900s, Labquality chose to seek certification for its operating system to support its quality control. Labquality has had a certified quality control system based on the standard SFS-EN ISO 9001 since 1996. The company’s operations also take into account the criteria stated in the above-mentioned ISO/IEC Guide 43-1:1996 and ILAC-G13:2008. Preparations for accreditation under ISO 17043 are under way.

The terminological situation

**Figure 1. Steroid and peptide hormones 4, 2007.**

The reference value for testosterone in the control sample was determined using the liquid chromatography-mass spectrometry reference method, LC-MS/MS [1]. Extended measurement uncertainty (U) determined in accordance with GUM [2] is 11.6 %. The sample was pooled female serum, and the concentration measured using the reference method was 0.85 nmol/l (n=6, s=0.03, CV%=3.4). The laboratories were asked to provide measurement uncertainty of their method (in percent) in connection with the testosterone results, as well as the method for determining measurement uncertainty.
is very confused in many countries. The terms accreditation and certification tend to be mixed up and they may be used to mean other concepts than in international ISO circles.

Choosing quality assessment surveys

Labquality offers wide selection of external quality assessment surveys, even within specialities. The programme for 2010 covers 157 schemes for nine specialties and includes 588 surveys. Those in need of quality assessment services have to analyse their needs and the supply of services available in order to make a decision. In Finland, participation in the surveys is voluntary in all specialties except microbiology, where authorisation is required.

Annex C of the ISO 17043 standard, which governs the operation of testing providers, also offers the laboratories instructions on choosing the surveys and points out factors to consider. The purpose of the annex is to promote harmonisation between different parties such as accreditation bodies and clients of laboratories. When EQA results are used to assess the performance of laboratories, it is essential that all parties are confident that the surveys are performed and arranged appropriately.

The parties should also have a clear picture of the criteria and performance assessments, and of how the results of these assessments are used, e.g. in accreditation. However, it is the responsibility of the laboratory to choose a reference measurement that enables its results to be correctly assessed.

Confidentiality and publicity

Laboratories participate in the surveys on a voluntary basis. The process and the reports for each laboratory are confidential. However, with the laboratory’s consent, some parties should, where necessary, have access to the external quality assessment results and to other detailed information concerning the whole process, such as the process and the grounds for determining the reference values, instructions for the survey, the statistical principles for processing and interpreting the data, and the final report of the reference measurement.

Survey organisation and participation frequency

The laboratory should assess the appropriateness of the chosen survey for the tests, procedures and standardisation it uses. Assessing how often the surveys are arranged and how often the laboratory should participate is very important for the laboratory. Naturally, both choice and participation are influenced by how often the surveys are available and by the level of quality and the related requirements determined by the laboratory itself. Finland does not have national guidelines for participation frequency, except for microbiology: according to guidelines issued by the authorisation committee of the National Institute for Health and Welfare (formerly the Finnish National Public Health Institute), a laboratory performing microbiological tests should participate in a survey four times a year.

The frequency with which proficiency tests are arranged for different specialties by different providers varies considerably (from once a year to 12–24 times a year). The European Committee for External Quality Assurance Programmes in Laboratory Medicine (EQALM) was established to consider how often reference measurements should be arranged from the
point of view of both the organisers and the participants, taking into account the state of the art of the analytics in question.

**Other points to consider**

In addition to the matters discussed above, laboratories should assess logistical aspects. Besides the date of the EQA survey, the stability of the control samples used and the number of participating laboratories should be taken into consideration. The criteria for good performance vary from one organiser to another, and they may be based on different views and needs. In Finland and in other Nordic countries the quality targets for external quality assessment surveys in laboratory medicine are strict in comparison with those used in Central Europe. The quality targets are set for educational purposes, and the aim of these strict targets is to raise the level of quality of the laboratories. The quality targets set for the total analytical error can be met if the analytical system is functioning faultlessly.

Different EQA providers offer surveys with very similar names but different contents, making price comparison difficult. The sample material used has a critical impact on the level of quality of the reference measurements. Access to materials such as patient samples has become much more difficult, while the prices of commercial samples have risen. Labquality aims to use samples similar to patient samples wherever they are available and whenever economically feasible. A good example of sample quality is the case where samples for certain virology surveys were provided from a single donor, thus allowing clinical case interpretation to be included in the survey. Lately, laboratories have indicated their preference for certified reference materials. Unfortunately, such material is available for very limited purposes, mainly for basic chemistry analytes.

The information content of the survey, readability and delivery time of the reports should also be assessed, when a laboratory compares the EQA organisers.

ISO 17043 will increase dialogue between EQA organisers and the laboratories participating in the surveys and promote their joint effort to improve the quality of analytics for the benefit of the patient.

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**REFERENCES**


ISO 17043
Microbiological point-of-care testing is completely different from chemical and haematological point-of-care testing. Microbiological testing—whether in a laboratory or at point-of-care— is used to detect pathogens in a patient’s sample. In other words, the test result should confirm the aetiological diagnosis of the disease: whether the patient has a disease caused by the suspected micro-organism or not. In this respect, microbiological point-of-care testing is completely different from chemical and haematological point-of-care testing, the aim of which is not to arrive at a diagnosis but to monitor a condition that has already been diagnosed, e.g. for purposes of treatment.

Infectious diseases can be treated with specific medicines that can shorten the duration of the illness, prevent exacerbation and reduce the patient’s infectiousness. For this reason, the treatment decision often has to be made quickly. Once the pathogen has been identified, treatment with antimicrobial medication can be targeted and maximum benefit can be obtained. For example, the treatment of influenza should be initiated within two days after the symptoms appear, so there is often no time for a microbiological laboratory diagnosis.

Careful consideration needed

Microbiological point-of-care tests are meant to shorten the delay of results associated with a specific diagnosis. At the moment, tests are available for the diagnosis of several micro-organisms. However, these point-of-care tests are always inferior to conventional laboratory methods in terms of clinical sensitivity and clinical specificity, and they are not necessarily cheaper. The status of these tests in diagnostics

- The status of microbiological POC tests should carefully be considered, says Antti Nissinen. PHOTO: ANITA LATVALA
should therefore be carefully considered, especially in the diagnosis of complex microorganisms such as HIV. Before a point-of-care test is adopted, careful consideration should be given to how the test will serve the unit concerned. At the emergency clinic, for example, point-of-care test results should be obtained while the patient waits and should influence the treatment decision. If this is not possible, a sample should be collected for conventional laboratory tests because of their superior quality. If the point-of-care test results cannot be relied on or the result is obtained too late, examinations may be duplicated, resulting in higher costs and possibly also conflicting test results.

Specificity and sensitivity

Microbiological point-of-care tests do not measure the levels of a certain substance in the sample but the presence or absence of a micro-organism, most commonly a micro-organism antigen. Both the sensitivity and the specificity of the test are nearly always below 100%. The predictive value of the point-of-care test result therefore depends greatly on the prevalence of the disease in the patient population examined. Let us assume that the sensitivity and specificity of the point-of-care test are high (99%). If the prevalence of the disease in the patient population examined is 10%, the positive predictive value of such a sensitive and specific test (91%) is quite good. But if the prevalence is only 1%, the positive predictive value falls to 50%. In other words, only one positive test result in two really is positive. It is therefore very important that microbiological point-of-care tests are only used in patients in whom the disease tested for is probable in relation to the patient’s medical history, clinical picture and other factors influencing the prevalence of the disease (the patient’s age and epidemiological situation). In the case of the *Streptococcus A* antigen test, for example, in order to obtain the best possible positive predictive value the test should only be performed on patients with the relevant symptoms, never as a screening test for every patient with symptoms of the upper airways.

"Microbiological point-of-care testing detects the pathogen causing the infectious disease."

Same regulations for POCT and laboratory tests

Microbiological point-of-care testing detects the pathogen causing the infectious disease. Therefore the same regulations apply to point-of-care testing as to the laboratory diagnosis of infectious diseases. In Finland, for example, microbiological point-of-care testing is subject to legal authorisation unlike other point-of-care testing. The Communicable Diseases Act stipulates where the diagnostic tests can be performed and who can authorise them. The Communicable Diseases Decree provides more specific regulations.

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CONTROLS FOR POINT-OF-CARE

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Controls for **POINT-OF-CARE** tests:

- CRP Controls and Calibrators
- EBV Controls
- Glucose Controls
- Hb Controls
- Liquid HbA1c Controls
- Streptococcus (group A) Antigen Controls
- Urine Dipstick Controls
- WBC Haematology Controls for HemoCue
- Blood Gas Controls
Virological tests used in outpatient care

JUKKA SUNI

The purpose of virological point-of-care tests is to evaluate the presence of either microbial antigen or, more rarely, antimicrobial antibodies. The most commonly used antigen tests are those used to diagnose an influenza A, influenza B or RSV infection. The most widely used virological point-of-care test, however, is the test for acute mononucleosis, usually measuring heterophile IgM antibodies.

Less frequently used tests include faecal rotavirus/adenovirus tests, an anti-HIV antibody test and a test measuring IgM antibodies against the Puumala hantavirus. New tests about to be launched include a norovirus test, an HIV Ag/Ab test and a test measuring specific anti-EBV antibodies in the diagnosis of mononucleosis.

The use of virological tests, as well as tests for infectious diseases on the whole, involves many problems not applicable to other point-of-care tests or their use:

- The use and monitoring of these tests comes under the purview of the Communicable Diseases Act and must be under the responsibility of a clinical microbiologist or hospital microbiologist.
- If a positive result is obtained, the laboratory should submit a communicable disease notification to the National Institute for Health and Welfare.
- The majority of tests are used seasonally in connection with epidemics. The extent of epidemics varies from year to year, which makes it difficult to assess the number of tests required.
- Changes in the virus antigen, as well as viral variants, may affect test sensitivity (as with the influenza A(H1N1) virus).
- A positive result may require verification by another method (e.g. HIV tests).
- Many tests are relatively insensitive, so a negative result is not 100% certain.

Virological point-of-care tests are used for the following purposes:

- early identification of epidemics (influenza A/B, RSV)
- patient cohorting (norovirus tests and rota/adenovirus tests)
- diagnostics (mononucleosis, Puumala virus infection)
- targeted screening tests, with certain restrictions (HIV).
Tick-borne encephalitis virus

ANU JÄÄSKELÄINEN, OLLI VAPALAHTI

Tick-borne encephalitis (TBE or Kumlange disease) is an infection caused by the TBE virus (a flavivirus) that can lead to serious CNS symptoms. Flaviviruses are small (diameter about 50 nm), spherical, enveloped viruses. Their genome is a single-stranded RNA, 10 to 11 kb, which functions as mRNA. The flavivirus particle consists of an envelope formed from cell lipid membranes and two virus envelope proteins (M, membrane, and E, envelope), and a protein shell consisting of a virus shell protein (C, capsid) inside the envelope. Innermost is the positive-stranded RNA which, having infected the cell, codes for the non-structural proteins 1–5 (NS1–5), one of which is a polymerase, in addition to the three structural proteins mentioned above.

The TBE virus (TBEV) is the only flavivirus occurring in Finland that is pathogenic to humans. Other important flaviviruses include the Japanese encephalitis, yellow fever, West Nile and dengue viruses, the latter causing dengue fever in more than 30 Finnish travellers to distant countries every year. These viruses may cross-react in serological tests, and the patient’s travel history may therefore play an important diagnostic role. There are three TBEV subtypes: European, Siberian and Far Eastern. The subtypes also cross-react in serological tests, and the same vaccine appears to protect against diseases caused by all subtypes.

Ecology and epidemiology

TBE occurs in a Eurasian zone from Europe to China and Japan. In 1990–2007, an average of 2,800 cases per year were reported in Europe, Russia excluded. In Russia the average number of reported cases per year was 6,000 during the same period (Süss, 2008). In Finland, 20 to 40 cases have been reported annually over the last few years.

The TBEV subtypes are mainly transmitted by various tick species. *Ixodes ricinus*, which occurs in Finland and other parts of Europe, transmits the European subtype, while the taiga tick *I. persulcatus*, whose range extends from Eastern Europe to the Far East, transmits the other two subtypes (Siberian and Far Eastern). *I. persulcatus* and the Siberian TBEV subtype spread by it have also been observed in the Kokkola archipelago off the west coast of Finland (Jääskeläinen et al., 2006). In the other traditionally TBE endemic areas of Finland (Åland islands and Turku archipelago, the Lappeenranta area and Isosaari island off Helsinki), only *I. ricinus* and the European TBEV subtype have been observed (Brummer-Korvenkontio et al., 1973; Han et al., 2001).

Unlike *Borrelia* bacteria, the other important pathogen transmitted by ticks, TBEV occurs in by no means all habitats of its vector. In order to persist in nature, the virus must be able to transfer from one generation of ticks to the next. The virus is readily transmitted from an infected tick to an uninfected one via the immune defence cells of a rodent as the ticks feed simultaneously on the skin of the same host (Labuda et al., 1996). Such simultaneous activity of different tick generations and the consequent preservation of TBEV in nature demands specific climatic conditions (Randolph et al., 2000). For this reason, TBE is much more common in Central Europe and the Baltic countries than in Northern Europe, where the disease is endemic only locally. In Finland, for example, about two
After hatching from an egg, a tick has three life stages: larva, nymph and adult. Here a female tick (*Ixodes ricinus*) is accompanied by two larvae and one nymph. Photo: Baxter.

![Image of ticks](image)

The progression of TBE with two phases, graph adapted from Holzmann (2003).

Infection weeks from infection

- neurological symptoms
  - IgM antibodies
  - IgG antibodies

Course of disease

Tick-borne encephalitis is usually transmitted by the bite of a TBEV-infected tick. However, the virus can withstand the harsh environment of the human alimentary tract, and thus can be transmitted through unpasteurised milk (notably goat’s milk) or unpasteurised milk products such as cheese. The seriousness of the disease ranges from asymptomatic or mild to serious encephalitis and meningitis. Typically, the disease has two phases. Infection is followed by 4 to 28 days of asymptomatic incubation period before the first phase manifests with flu-like general symptoms such as fever, nausea and headache. The first phase lasts 2 to 10 days and is followed by an asymptomatic period of about a week before the second phase begins with the actual CNS symptoms, which develop only in about one-third of acute TBE cases used to originate from the Åland Islands, which have a population of only some 25,000. A vaccination campaign has changed the situation slightly, with fewer cases subsequently reported in Åland. The prevalence areas of TBE may change due to climate change (Randolph & Rogers, 2000), and there is already evidence that the disease has spread to new areas in Finland, with one case in Närpiö in 2007, one in Varkaus in 2008, and several cases in Simo, Lapland, in 2008 and 2009 (National Institute for Health and Welfare, infectious diseases register).
of infected persons. The disease can be manifested as meningitis, meningoencephalitis or meningoencephalomyelitis. The mildest cases present with headache and fever. Decreased level of consciousness is seen in about one-third of patients. The extremities or respiratory organs may be paralysed, and some patients require intensive care. The material from Åland shows a slightly milder form of the disease, perhaps because milder infections are more easily diagnosed in the region. Wahlberg et al. (2006) reviewed all 301 TBE cases from Åland between 1959 and 2005. Of these patients, 84% had a temperature of over 39 °C, but pareses occurred in 4% only, and 1% required mechanical ventilation. The mortality rate from tick-borne encephalitis is below 2% in Europe, but in Asian Russia it may be as high as several dozens of percents. It is not completely clear whether the difference is due to the different TBEV subtypes – the Siberian subtype appears to cause chronic infections more often than the other types (Gritsun et al., 2003), while the Far Eastern subtype appears more serious than the others – or to potential differences in the health care systems in Western Europe and Eastern Siberia. The risk of developing a serious case of TBE increases with age. (From the review article by Lindquist & Vapalahti, 2008.)

**Diagnosis**

The symptoms of tick-borne encephalitis are non-specific, and the clinical picture may match that of many other infections. This means that only a laboratory-confirmed diagnosis is reliable. The diagnosis of tick-borne encephalitis is based on serum antibody determination. In the first phase of the disease, nucleic acid determination methods could in principle be used in diagnosis (and these are actually used in some European countries) (Donoso Mantke et al., 2008), but this is only useful in rare cases, because the first phase is often too mild for the patient to seek medical help. When the actual CNS symptoms appear in the second phase of the disease, IgM and often also IgG antibodies can be detected in the serum, but the virus is no longer present in the blood or cerebrospinal fluid (CSF), and the determination of viral nucleic acid or viral antigen or cultivation of the virus is often not possible in this phase. ELISA kits are widely used for antibody determination, and for example, Helsinki University Central Hospital Laboratory (HUSLAB) utilizes IgM ELISA based on a recombinant antigen (Jääskeläinen et al., 2003). TBE antibodies can be seen also in the CSF in only about half of the patients at the start of acute neurological TBE symptoms. Within ten days of the beginning of the second phase, all TBE patients present with antibodies in the CSF (Holzmann 2003).

Another widely used test is the haemagglutination inhibition (HI) test. Instead of differentiating between antibody classes, this test measures the total antibody levels. A diagnostic increase in antibody titres between paired sera is required to diagnose acute TBE with the HI test.

Flaviviruses cross-react in antibody tests. In Finland, a flavivirus infection other than TBE would be likely to occur mainly in travellers. A TBE vaccine response is also seen in the antibody tests, for the determination of which ELISA is more sensitive than HI. However, the IgM responses are weak and transient. In theory, vaccines against other flaviviruses could also cross-react in a TBE antibody test. At the moment, there is no serological test available that could differentiate between antibodies against the various TBEV subtypes.

IgM antibodies are present in the serum for several months after the infection. IgG antibodies persist for the patient’s lifetime and protect against reinfection.

**Prevention**

There is no specific treatment for tick-borne encephalitis. However, the risk of infection can be reduced by avoiding tick bites in endemic areas.
or by vaccination. TBEV is transmitted from tick saliva within a few minutes after a tick bite. However, often ticks do not attach themselves immediately but instead may move around in the clothing and on the skin for a considerable time, looking for a suitable spot. On the other hand, the other important tick-borne disease, borreliosis, is usually transmitted to humans only after the tick has been attached for several hours or even two days. Thus it is a good idea to check the skin in the summer after being outside in a tick habitat, and to remove any ticks before or after they become attached. It should also be borne in mind that a tick check does not offer much protection against TBE. Protective clothing and tick repel lents may be useful when walking in tick habitats.

There is a formalin-inactivated vaccine against TBE based on complete virus particles. In Finland, vaccines based on the same principles are available from two manufacturers: Ticovac from Baxter and Encepur from Novartis. The basic vaccination series comprises two doses with a one-month interval and a third vaccination after about one year. Åland is one area where a clear reduction in the number of cases has been achieved following the start of a general vaccination programme in 2006. However, booster vaccinations are necessary at least every five years for continued protection. Even though the vaccine is effective, TBE may, in rare cases, be suspected in a vaccinated person. In the 2000s, 27 cases have been reported in Sweden where a patient had developed TBE despite a previous vaccination series (Andersson et al., 2010). In such a case, IgG antibody titres are high in the very first samples, while IgM may be low or even negative. Another serum sample and antibody determination from the CSF may be useful. Once a person has had TBE, the immunity lasts a lifetime.

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Lotta Joutsi-Korhonen predicts changes and challenges in laboratory work with the new pharmacotherapeutic practices.

PHOTO: MINNA SEPPÄ

Monitoring of anticoagulant effects is essential to ensure safe and effective therapy. Traditionally vitamin K antagonists, such as warfarin, require repeated monitoring to obtain therapeutic control, whereas some new drugs require monitoring only in special situations.

Antithrombotic pharmacotherapy has been a major field of research and development already for several years its main aim to achieve an effective, safe and easy-to-use anticoagulant. Ideally, laboratory monitoring of the drug response would not be necessary. However, laboratory tests will be needed in case of complications or other special medical situations of patient care.
Generally, the assessment of therapeutic response is used to achieve and maintain safe and effective treatment. The monitoring of anticoagulant effects supports successful therapeutic control both during a stable phase and prevention or management of complications (e.g. in case of ineffective treatment, in association with surgical procedures or acute bleeds). It is mandatory to define a suitable method and to establish the target therapeutic (and prophylactic) range for each pharmaceutical agent. The effects on different coagulation tests should also be recognized. The monitoring requirements of different drug agents vary greatly. Traditionally vitamin K antagonists, such as warfarin, require repeated monitoring in every patient to obtain therapeutic control, whereas some drugs require monitoring only under special situations and/or in special patients.

At the moment, in Finland warfarin is the only oral anticoagulant approved for long-term therapy. Repeated INR monitoring is required to achieve and maintain therapeutic control, whereas some drugs require monitoring only under special situations and/or in special patients.

The direct FXa inhibitor rivaroxaban and the direct thrombin inhibitor dabigatran etexilate, both novel antithrombotic agents are designed to replace warfarin as oral anticoagulant. The have been shown to influence aPTT and prothrombin time values, but these tests are not reliable for dose-response evaluation. Injectable fondaparinux is an antithrombin-dependent FXa inhibitor, of which the therapeutic doses have no significant effect on standard coagulation tests, but the anti-FXa effect can be monitored with proper standards. The parenteral thrombin inhibitors lepirudin and bivalirudin are known to accumulate, especially in cases of renal insufficiency. The currently used aPTT monitoring methods may misestimate their dose-responses and thus predispose to either lepirudin under- or overdosing. Laboratory methods such as ACT (activated clotting time), PiCT® (prothrombin induced clotting time) and those based on thrombin time and ecarin activation as well as chromogenic anti-FIIa methods have been developed as monitoring tools.

Monitoring of anticoagulant effects is essential in certain patient groups and/or in complicated situations to ensure safe and effective pharmacotherapy. The therapeutic indications for novel anticoagulants are broadening, and pharmaceutical agents already on the market are encountering new competitors. Changing clinical practices also mean changes and challenges for the laboratory work. Interaction between the laboratory and clinic is necessary in managing these changes.

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Haemolysis means the breakdown of erythrocytes (red blood cells). In haemolytic conditions, the breakdown of erythrocytes is increased and their lifespan is reduced. Since the bone marrow is able to increase its erythrocyte production by 6- to 10-fold, a reduced erythrocyte lifespan does not necessarily result in anaemia. It is only when the lifespan becomes shorter than 15 to 20 days that erythrocyte breakdown exceeds the production capacity and results in anaemia.

The aetiology of haemolytic conditions

Haemolytic conditions may be hereditary or due to an acquired disease. Hereditary haemolytic conditions may involve structural defects in the erythrocyte membrane, an abnormality in their energy metabolism or a defect in haemoglobin molecule structure. Acquired haemolytic conditions may be due to erythrocyte antibodies (immunological haemolysis) or increased mechanical wear and tear (fragmentation haemolysis). Haemolysis may be acute or chronic, and chronic haemolytic conditions may also involve acute exacerbations. Haemolysis may take place inside the blood vessels (intravascular haemolysis) or outside them, e.g. in the macrophages of the liver, spleen or bone marrow (extravascular haemolysis).

Laboratory tests

The laboratory diagnosis of haemolysis is based on demonstrating increased erythrocyte production, measuring haemoglobin levels as well as degradation and metabolism products in blood or urine, assessing erythrocyte morphology and properties and detecting antibodies attached to the erythrocyte membrane. Diagnosis aims both to confirm the presence of haemolysis and to identify the mechanism and aetiology involved. The most common laboratory tests for haemolytic conditions include the blood reticulocyte count, plasma or serum levels of bilirubin, haemoglobin and lactate dehydrogenase (LDH), direct Coombs test (also known as the direct antiglobulin test) and peripheral blood smears (blood cell morphology).

Blood counts

The number of reticulocytes, or young erythrocytes, can be reliably assessed with modern blood count analysers. The absolute reticulocyte count and the reticulocyte-erythrocyte ratio are almost always elevated in patients with haemolysis. This is because the body strives to compensate for increased
erythrocyte breakdown by increasing its erythrocyte production. In patients with normal bone marrow function, increased reticulocyte formation can be observed 3 to 5 days after the onset of haemolysis.

Clinical chemistry tests

Increased erythrocyte breakdown typically results in increased free bilirubin and LDH in plasma and in reduced haptoglobin levels. As erythrocytes break down, LDH and haemoglobin are released in the bloodstream. LDH is a common intracellular enzyme, found abundantly in erythrocytes as well as in the myocardium, skeletal muscle, liver, pancreas and lungs. Increased LDH levels are therefore also observed in many types of tissue damage and in megaloblastic anaemia. The breakdown of haem in haemoglobin results in the formation of free bilirubin. The liver conjugates this with glucuronic acid and excretes the conjugated bilirubin in the bile. A healthy liver is able to eliminate about three times the normal quantity of bilirubin. The level of free, unconjugated bilirubin in plasma. The resulting complex is transferred to the reticuloendothelial cells in the liver for degradation. Patients with haemolytic conditions therefore have low or undetectable haptoglobin levels. Haptoglobin levels remain reduced for about a week after each haemolytic episode. Reduced haptoglobin levels may also occur in patients with impaired hepatic function. Haptoglobin is a so-called acute phase protein, and its levels may increase two-to-threefold in connection with inflammation or tissue damage. This should be remembered when interpreting haptoglobin test results.

Antibodies

The direct Coombs test is used to identify the presence of immunological haemolysis. It detects the presence of antibodies or complement proteins attached to the patient’s erythrocyte membrane in the bloodstream. The sample is usually first tested with a polyspecific antiglobulin reagent containing antibodies against both human IgG and complement components. If this yields a positive result, separate tests for IgG antibodies and the complement component C3d are performed using monospecific reagents. Warm antibodies that react strongly at +37 °C are usually IgG antibodies. Cold agglutination conditions usually only yield a positive reaction to anti-complement antibodies.

Microscopic examination of a blood smear

A peripheral blood smear may be used to detect findings typical of haemolysis and to obtain an indication of the mechanism involved. Increased erythrocyte formation presents as polychromasia, i.e. an increase in blue-staining reticulocytes. Increased LDH and haemoglobin are released in the bloodstream. LDH is a common intracellular enzyme, found abundantly in erythrocytes as well as in the myocardium, skeletal muscle, liver, pancreas and lungs. Increased LDH levels are therefore also observed in many types of tissue damage and in megaloblastic anaemia. The breakdown of haem in haemoglobin results in the formation of free bilirubin. The liver conjugates this with glucuronic acid and excretes the conjugated bilirubin in the bile. A healthy liver is able to eliminate about three times the normal quantity of bilirubin. The level of free, unconjugated bilirubin in plasma. The resulting complex is transferred to the reticuloendothelial cells in the liver for degradation. Patients with haemolytic conditions therefore have low or undetectable haptoglobin levels. Haptoglobin levels remain reduced for about a week after each haemolytic episode. Reduced haptoglobin levels may also occur in patients with impaired hepatic function. Haptoglobin is a so-called acute phase protein, and its levels may increase two-to-threefold in connection with inflammation or tissue damage. This should be remembered when interpreting haptoglobin test results.

[210x627]"Haemolysis may take place inside the blood vessels or outside them."
small, spherical erythrocytes. Spherocytes are also observed in hereditary spherocytosis (a structural defect in the cell membrane), in which they are a characteristic finding. Typical morphological findings are also seen in other hereditary structural cell membrane defects (elliptocytes in hereditary elliptocytosis; poikilocytosis, e.g. elliptocytes, spherocytes and erythrocyte fragments, in hereditary pyropoikilocytosis). In suspected thalassaemia or haemoglobinopathy (e.g. sickle cell anaemia), findings on erythrocyte morphology may help to confirm the diagnosis. In fragmentation haemolysis, associated with e.g. microangiopathic vascular damage or an artificial heart valve, erythrocyte fragments known as schistocytes can be seen in the smear.

**Urine assays**

In severe intravascular haemolysis, haemoglobin may exceed the binding capacity of haptoglobin. Haemoglobin can then be detected in plasma and urine. Haemoglobin disappears from plasma and urine in a few hours once the intravascular haemolysis has stopped. In severe intravascular haemolysis, some of the haemoglobin in renal glomerular filtrate is absorbed and broken down in tubular cells. The released iron binds with haemosiderin. Haemosiderin-containing tubular cells are shed in the urine, and an iron stain of urinary sediment may therefore indicate the presence of haemosiderin a few days after the onset of intravascular haemolysis. Urine haemosiderin tests may be positive for at least a week after the end of intravascular haemolysis.

**Specific assays**

More specific assays may be used to confirm the presence of several haemolytic conditions, particularly hereditary ones. When diagnosing hereditary spherocytosis, flow cytometric tests and glycerol haemolysis assays may be used in addition to morphological findings. When suspecting alpha or beta thalassaemia, an alpha thalassaemia mutation test or a haemoglobin fraction analysis, respectively, can be performed on the blood sample. In patients with suspected haemoglobinopathy, isoelectric focusing of haemoglobin is recommended. When paroxysmal nocturnal haemoglobinuria (PNH) is suspected, further information can be obtained by detecting the PNH clone in leukocytes or erythrocytes with flow cytometry.

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Elements in laboratory process quality

A quality system based on international standards is an excellent tool for developing and managing laboratory process quality.

The quality of a laboratory process is a large entity consisting of several elements. Initially, qualitative thinking usually involved just the laboratory analysis and the result obtained from it. Today, it is understood that several other factors affecting the total quality of a laboratory process, both before and after the analysis, should also be taken into account. The most important factors influencing the outcome, aside from the analytical method, culture media, reagents and equipment used, also include the expertise of the staff and the quality of the sample.
Pre-analytics

High-quality analysis results always require high-quality samples. Samples should be collected at the right time, from the right place and in the right way. It is also important that samples are stored appropriately and taken to the laboratory as quickly as possible. A request for analysis, together with all the necessary information, should be submitted with the sample.

The laboratory should ensure that a laboratory analysis manual with specific, up-to-date information to ensure appropriate sample collection is available to all sample collectors. In an electronic manual, any necessary changes can be made in real time.

Staff

High-quality laboratory results require competent staff. Laboratory staff should have appropriate education and training and have sufficient expertise in the work they are required to do.

Constant maintenance of professional skills is important because methods change and develop. This can be achieved through internal training organised by the laboratory and through participation in educational events organised by outside bodies. Each assignment should be preceded by a practical introduction by a colleague already familiar with the task concerned.

Staff should also be informed of the person to whom to turn to if any problems arise. Exactly who is responsible for what should be clear to all.

Equipment and methods

The introduction of new equipment and methods requires the appropriate validation or verification of the equipment or method concerned. Such validation or verification must be sufficient to ensure the accuracy and repeatability of the results. However, there is no universally applicable definition of what is sufficient, and the size and quality of the samples to be examined should be defined on a case-to-case basis.

Methods already in use can be updated by keeping abreast of developments in the field. Occupational safety and environmental matters are becoming increasingly pertinent, and more attention should be given to them when selecting new methods and reviewing old practices.

Internal and external quality control

In Finland, laboratory methods have long been scrutinised using external quality control samples, often obtained from the national body Labquality. Ordering quality control samples from Labquality is easy and the samples are well suited for laboratory methods.

Internal quality control began to receive closer attention as quality systems became more common. Internal quality control monitors factors such as the functionality of equipment, culture media and reagents and,
at best, the functionality of the whole process. Any abnormal results require instant attention, together with action to rectify the cause.

**Instructions and documentation**

To ensure the accuracy and repeatability of laboratory results, analyses must always be performed in accordance with the agreed procedure. This requires the compilation and maintenance of written working instructions. When everything is carried out according to written instructions, there will be no deviations from the agreed working procedures and each analysis will always be performed in the same way.

Written documentation is important even though it may sometimes feel laborious. The information that should be recorded includes staff training and work introduction, the results and reports of internal and external quality control, method and equipment validations, equipment maintenance, and any exceptional situations and the actions taken to rectify them.

The staff should have clear instructions on document archiving, including the archiving method and time.

**Post-analytics**

Post-analytics is that part of the laboratory process over which the laboratory has least control. What happens to a correct analysis result as such. Does it reach its destination in the correct form quickly enough and is it understood in the right way? Does the laboratory result influence the patient’s treatment in the right way? Where does the laboratory's responsibility end?

Electronic data exchange is an everyday part of activities for most laboratories. When data are transferred to another system, something unexpected may happen in the transition. Feedback on these problems is most likely to be received if the recipient of the result has to pay extra costs. Situations in which the result given by the laboratory needs to be corrected afterwards for one reason or another are especially problematic. Does the corrected result reach the doctor concerned or does the original inaccurate result remain valid?

The most important results can be reported fastest by phone (e.g. positive blood culture and cerebrospinal fluid findings). The impact and the effect of the result on the patient's treatment can be discussed at the same time. However, only a small percentage of laboratory results can be accompanied by personal consultations.

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